The Effect of Space Radiation on the Induction of Chromosome Damage

K. George[1], H. Wu[2], V. Willingham[1] and F. Cucinotta[3]

[1]Wyle Laboratories, Houston, TX. [2]Kelsey-Seybold, Houston, TX. [3] NASA, Johnson Space Center, Houston, TX.

To obtain information on the cytogenetic damage caused by space radiation, chromosome exchanges in lymphocytes from crewmembers of long-term Mir missions, and a shorter duration shuttle mission, were examined using fluorescence in situ hybridization. A significant increase in chromosomal aberrations was observed after the long duration flights. The ratio of aberrations identified as complex was higher post-flight for some crewmembers, which is thought to be an indication of exposure to high-LET radiation. Ground-based studies have shown that the frequency of aberrations measured post-flight could be influenced by a mitotic delay in cells damaged by high-LET radiation and this effect could lower biological dose estimates. To counteract this effect, prematurely condensed chromosome (PCC) spreads were collected. Frequencies of aberrations in PCC were compared with those in metaphase spreads.

Key words (Biodosimetry, chromosomes, high-LET, FISH)

Introduction

As astronauts spend longer periods in space during International Space Station missions their radiation exposures will come close to currently accepted annual limits, and it has therefore become important to measure radiation doses and assess risk to individuals much more accurately than in past short-duration Space Shuttle flights.

Physical doses are measured during flight using passive thermo luminescent detectors, and plastic nuclear track detectors. These measurements provide an accumulated absorbed skin dose. An increase in chromosome damage in human lymphocyte cells is linked to both prior exposure and risk (1) and can easily measured using fluorescence in situ hybridization (FISH) with whole chromosome probes. Biodosimetric measurements from chromosome damage provide direct information on biological response to radiation exposure in the altered gravity environment that can be combined with physical measurements, thus leading to better estimation of risk.

Ground based accelerator studies have clearly demonstrated (2) that elevated chromosomal damage is induced by heavy ions relative to x-rays or gamma rays, and that exposure to high-LET particles induces a higher ratio of complex type chromosome rearrangements resulting in a possible signature of this type of radiation exposure (3). Although the contribution from heavy ions is only a few percent of the dose received in space, the biological effect could be significant.

Studies of dicentric chromosomes, rings, fragments and chromatid aberrations assessed using Giemsa stained chromosome spreads from peripheral lymphocytes of crewmembers of ANTRRES, ALTAIR and EUROMIR missions (4,5) showed elevated frequencies of aberrations after orbital flights of more than 3 weeks. Our previous study using FISH analysis in lymphocytes from three crewmembers of a 115-day NASA-Mir mission (6) showed significant increases in chromosome damage post-flight. The present study includes analysis from three additional crewmembers of Mir missions and a closer examination of the contribution of complex type chromosome rearrangements is included. The effect of mitotic delay on the expression of high-LET radiation induced damage is also investigated in a ground based accelerator study and in astronaut lymphocytes after a short duration mission.

Materials and Method

1. *Biodosimetry*

Blood samples from crewmembers were collected in tubes containing sodium heparin. All samples were incubated in growth medium supplemented with PHA and metaphase spreads were harvested approximately 48 hours later after a two hour treatment with colcemid. PCC were collected after incubating cells with 50nM of calyculin A (Wako chemicals) for 30 minutes.

Chromosomes were hybridized *in situ* with fluorescence whole chromosome probes (Vysis) in a combination of spectrum orange and green using standard procedures. Unlabelled chromosomes were counterstained with DAPI and chromosome exchanges were analyzed using a Ziess fluorescence microscope.

2. *Ground studies*

Whole blood was irradiated with 1 GeV/u iron particles (LET of 140 KeV/μ) accelerated by the alternating gradient synchrotron at Brookhaven National Laboratory at a dose rate of 1 Gy/min. Lymphocytes were set up in culture immediately after irradiation. Metaphase spreads were collected at 4 times points after 48 – 84 hours in culture and PCC were collected after 48 and 72 hours in culture.

Spreads were prepared using a combination of FISH chromosome painting and harlequin staining achieved by incubating slides in 1 μg/ml Hoechst 33258 and exposing them to UV light. The spreads were dehydrated and dried for 1 hour at 55°C before applying fluorescence whole chromosome paint probes using standard procedures. After counterstaining with DAPI, second mitosis cells showed a harlequin pattern with darkly and brightly stained chromatids. Cells at first mitosis showed homogeneously stained chromatids. Chromosome exchanges were assessed in spreads in first division at each collection time.

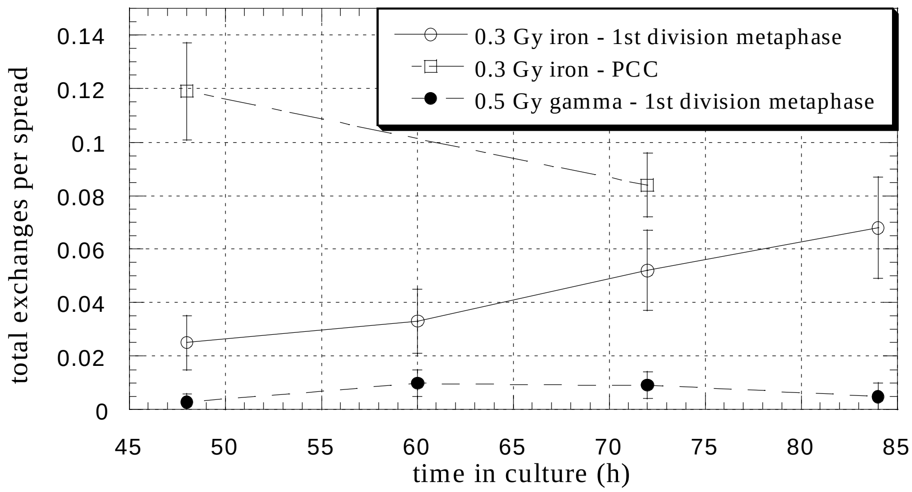
Results

Chromosome exchange frequencies measured in lymphocytes drawn from crewmembers before and after Mir missions of approximately 3 months are shown in table 1. Incomplete translocations were included with apparently simple reciprocal translocations, assuming that in most cases the reciprocal fragments are not visible (7). Complex-type exchanges are defined as an exchange involving 3 or more breaks in two or more chromosomes (8). When values for total exchanges in the individual chromosomes were extrapolated to the whole genome using the formula of Lucas et al. (9), it was apparent that frequencies increased post-flight for all crewmembers. Although the number of complex exchanges detected was quiet low, two crewmembers lymphocytes contained elevated frequencies of complex-type exchanges post-flight.

Figure 1 shows the delay in expression of chromosome damage in human lymphocytes after in vitro irradiation with 0.3 Gy of 1 GeV/u iron particles. The frequency of exchanges in chromosomes 2 and 1 measured in spreads at first division after exposure increases approximately 3 fold when comparing samples collected at 48 hours with those collected after 84 hours. No change in the frequency of exchanges is seen in first division cells collected at different times after irradiation with gamma rays. In PCC samples it was not possible to determine the cell division using the harlequin staining method due to limitations in the resolution of the chromatids in these spreads. Therefore, exchanges were measured in all spreads in each sample. Frequencies of total chromosome exchanges in PCC, shown in figure 1, decreased with incubation time when comparing samples collected after 48 and 72 hours.

A comparison of chromosome exchange frequencies measured in PCC and metaphase spreads from lymphocytes drawn from two crewmembers before and after a 7 day shuttle mission are shown in table 2. The frequency of aberrations pre-flight is similar in both PCC and metaphase spreads. However, for one crewmember the post-flight frequency of exchanges is higher in PCC samples than metaphase spreads.

Figure 1 - frequency of total exchanges measured in chromosomes 2 and 1 after exposure to iron particles and chromosomes 2 and 5 after exposure to gamma rays.



Conclusions

The frequency of total chromosome exchanges increased in lymphocytes from all 6 crewmembers after long duration (approximately 3 month) Mir missions. For two crewmembers, the ratio of complex to simple exchanges was considerably higher post-flight than preflight. 7 of the 41 exchanges found post-flight for crewmember 1 were complex, and 6 of 25 exchanges were complex for crewmember 3. Since complex aberrations are induced more frequently by high-LET radiation, this increase could represent a signature of exposure to heavy ions in-flight.

Ground studies of lymphocytes irradiated in vitro with 1 GeV/u iron particles clearly show a delay in the expression of chromosome damage in cells undergoing first division after exposure, but no delay was evident for gamma irradiated cells. These data could indicate that post-flight biodosimetry samples could be influenced by a complicated pattern of mitotic delay after exposure to the mixed radiation field encountered in space. Frequencies of iron induced chromosome exchanges in PCC collected after a 48 and 72 hour incubation were higher than values obtained in metaphase spreads collected using standard colemid treatments. Calyculin A efficiently condenses all the lymphocyte chromosomes in the G2 and metaphase stages of the cell cycle. The difference in values obtained for metaphase and PCC could therefore be attributed to a prolonged cell cycle delay preventing cells from reaching mitosis.

Frequencies of exchanges in PCC collected after 48 hours were much higher than in PCC collected after 72 hours. Exchanges were measured in all PCC spreads regardless of cell cycle. Since most cells collected 72 hours after irradiation would have divided, this would explain the decrease in the frequency of exchanges in PCC collected at this later time.

Chromosome analysis in lymphocytes of two crewmembers of a short duration mission showed no difference when comparing pre-flight exchange frequencies measured in PCC and metaphase. However, for one crewmember the post-flight PCC sample contained significantly more exchanges than the metaphase sample. This increase in total exchanges in PCC post-flight was due to an increase in complex exchanges. Frequencies of simple translocations were similar post-flight in metaphase and PCC samples. Therefore, it would appear that the expression of chromosome damage measured after exposure to space radiation is affected by mitotic delay, especially the highly damaged cells containing complex chromosome rearrangements. PCC analysis is recommended for biodosimetry analysis to counteract these delays.

Acknowledgements

We are grateful to the staff of the Brookhaven National Laboratory for supporting the iron run and Dr J. Miller and colleagues at Lawrence Berkeley Laboratory for performing dosimetry measurements.

References

(1) Edwards, A.A., The use of chromosomal aberrations in human lymphocytes for biological dosimetry. Radiat. Res. 148, S39-44, 1997.

(2) Wu. H., Durante, M., George, K., and Yang, T.C., Induction of chromosome aberrations in human cells by charged particles. Radiat. Res. 148, S102-7, 1997

(3) Anderson, R.M., Marsden, S.J., Wright, E.G., Kadhim, M.A., Goodhead, D.T., and Griffin, C.S. Complex chromosome aberrations in peripheral blood lymphocytes as a potential biomarker of exposure to high-LET alpha-particles. Int. J. Radiat. Bio, 76, 31-40, 2000.

(4) Testard, I., Ricoul, M., Hoffschir, F., Flury-Herard, A., Dutrillaux, B., Federenko B., Gerasimenko, V., and Sabatier, L. Radiation-induced chromosome damage in astronauts' lymphocytes. Int. J. Radiat. Bio, 70, 403-411, 1996.

(5) Obe, G. Johannes, I., Johannes, C., Hallman, K., Reitz, G., and Facius, R. Chromosomal aberrations in blood lymphocytes of astronauts after long-term space flights. Int. J Radiat. Bio. 1997

(6) Yang, T.C., George, K., Johnson, A.S., Durante, M., and Federenko, B.S. Biodosimetry results from space flight Mir-18. Radiat. Res. 148, S17-S23, 1997.

(7) Wu, H., George, K., and Yang, T.C. Estimate of true incomplete exchanges using fluorescence *in situ* hybridization with telomere probes. Inter. J. Radiat. Bio. 73, 521-528, 1998.

(8) Savage. J.R.K., and Simpson, P.J. On scoring of FISH-'painted' chromosome-type exchange aberrations. Mutation Res. 307, 345-353. 1994.

(9) Lucas, J.N., Awa, A., Straume, T., Poggensee, M., Kodama, Y., Nakano, M., Ohtaki, K., Weier, U., Pinkel, D.,Gray, J., and Littlefield, G. Rapid translocation frequency analysis in humans decades after exposure to ionizing radiation. Int. J. Radiat. Bio. 62, 53-63, 1992.

Table 1 - chromosome aberrations measured pre- and post-flight for six crewmembers of long duration Mir missions.

Crew-member	Sample Collection	Cells scored	Chromosomes analyzed	Apparently simple translocations		Complex exchanges		Total exchanges		Values for total exchanges extrapolated to the whole genome (Freq. x 10^{-3})
				No.	Freq. ± SD ($x10^{-3}$)	No.	Freq. ± SD ($x10^{-3}$)	No.	Freq. ± SD ($x10^{-3}$)	
1	Pre-flight	4381	1 + 2	19	4.3 ± 1.0	1	0.2 ± 0.2	24	5.4 ± 1.1	18.9
	10 days after mission	6556	1 + 2	27	4.1 ± 0.8	7	1.1 ± 0.4	41	6.2 ± 1.0	21.7
	Pre-flight	1884	1,2 + 4	6	3.2 ± 1.3	1	0.5 ± 0.5	7	3.7 ± 1.4	9.6
	12 days after mission	4677	2 + 1	20	4.3 ± 1.0	2	0.4 ± 0.4	23	4.9 ± 1.0	17.1
3	Pre-flight	3995	2 +4	4	1.0 ± 0.5	0	0	4	1.0 ± 0.5	3.8
	Day of return	3553	2 + 1	18	6.8 ± 1.2	6	1.7 ± 0.7	25	7.0 ± 1.4	24.5
	240 days after mission	4745	2 + 1	14	2.9 ± 0.8	2	0.4 ± 0.3	18	3.8 ± 0.9	13.3
4	Pre-flight	3792	2 + 4	12	3.2 ± 0.9	3	0.8 ± 0.5	17	4.5 ± 1.1	17.1
	9 days after mission	4843	2 + 4	30	6.2 ± 1.1	3	0.6 ± 0.4	38	7.8 ± 1.3	29.6
	114 days after mission	3604	2 + 4	20	5.5 ± 1.2	0	0	23	6.4 ± 1.3	24.3
5	Pre-flight	742	2 + 4	3	4.0 ± 2.3	2	2.7 ± 1.9	3	6.7 ± 3.0	25.5
	9 days after mission	2630	2 + 4	19	7.2 ± 1.7	0	0	21	8.0 ± 1.7	30.4
6	Pre-flight	2852	2 + 4	7	2.4 ± 0.9	1	0.4 ± 0.4	8	2.8 ± 1.0	10.6
	Day of return	4672	2 + 4	26	5.6 ± 1.1	1	0.2 ± 0.2	30	6.4 ± 1.2	24.3
	9 days after mission	3147	2 + 4	13	4.1 ± 1.1	1	0.3 ± 0.3	19	6.0 ± 1.4	22.8

Table 2 – comparison of aberrations measured in PCC and metaphase pre- and post-flight for two crewmembers of short duration mission. Chromosomes 1 (orange), 2 (green) and 5(yellow) were analyzed in all samples.

Crew-member	Sample		Cells scored	Apparently simple translocations		Complex exchanges		Total exchanges	
				No.	Freq. ± SD ($x10^{-3}$)	No.	Freq. ± SD ($x10^{-3}$)	No.	Freq. ± SD ($x10^{-3}$)
1	Pre-flight	PCC	4766	10	2.1 ± 0.7	1	0.2 ± 0.2	16	3.4 ± 0.8
		Metaphase	2962	5	1.7 ± 0.7	1	0.3 ± 0.3	7	2.4 ± 0.9
	Day of return	PCC	5076	10	2.0 ± 0.6	1	0.2 ± 0.2	12	2.4 ± 0.7
		Metaphase	4287	7	1.6 ± 0.6	1	0.2 ± 0.2	10	2.3 ± 0.7
2	Pre-flight	PCC	993	0	0	0	0	0	0
		Metaphase	712	1	1.4 ± 1.4	0	0	1	1.4 ± 1.4
	Day of return	PCC	2549	4	1.6 ± 0.8	0	0	4	1.6 ± 0.8
		Metaphase	2529	4	1.6 ± 0.8	3	1.2 ± 0.7	7	2.8 ± 1.0